

a³
 wcl.
occurring mammalian thrombopoietin, wherein the DNA segment comprises a region that is at least 80% identical to the region between nucleotides number 237 and 722 of SEQ ID NO:1, and wherein said thrombopoietin stimulates MPL-dependent cell proliferation; and
a transcription terminator.

a⁴
 22. (amended) An expression vector according to claim 20 wherein said DNA segment comprises nucleotide 237 to nucleotide 722 of SEQ ID NO: 1 [or nucleotide 64 to nucleotide 525 of SEQ ID NO: 18].

✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓
 Please cancel claims 12, 14, 15, 16, 17, 18, 19, 21, 25, 26, and 37 without prejudice.

REMARKS

The undersigned representative thanks the Examiner for her courtesy in conducting an interview on August 1, 1995.

Entry of the above amendments is earnestly requested. With these amendments claims 10, 11, 13, 20, 22-24, 27, 28, 32 and 33 are now in this case. Claims 12, 14-19, 21, 25, 26 and 37 have been canceled. Claims 10, 11, 13, 20 and 22 have been amended. Applicants reserve the right to prosecute the canceled subject matter in one or more continuing applications.

The above amendments have been made to accelerate prosecution on certain preferred embodiments of Applicants' invention. In addition, the specification has been amended to correct a clerical error. No new matter has been added.

Claims 10 and 20 have been amended to recite that the polynucleotide or DNA segment, respectively, encodes naturally occurring mammalian thrombopoietin, wherein the polynucleotide or DNA segment comprises a region that is at least 80% identical to the region between nucleotides number

237 and 722 of SEQ ID NO:1, and wherein said thrombopoietin stimulates MPL-dependent cell proliferation. Claim 11 has been amended to recite "nucleotide 722". Claim 13 has been amended to recite "amino acid residue 206". Support for these limitations is found within Applicants' specification, such as at page 5.

Applicants have disclosed representative clones of naturally occurring mouse and human thrombopoietins, including their sequences, and have deposited representative clones with the American Type Culture Collection. Applicants have further disclosed methods for using these clones and sequence information obtained therefrom in cloning other mammalian thrombopoietin-encoding polynucleotides. See, for example, Applicants' specification at pages 11-13, and Example IX. Briefly, one prepares a cDNA library from mRNA obtained from a tissue shown to contain mRNA encoding TPO, such as lung, liver, heart, skeletal muscle, or kidney. These tissues are readily available in the art. The library is then probed with one of the disclosed DNAs or a fragment thereof, or with one or more small probes based on the disclosed sequences. Such methods are routine in the art, and are disclosed in detail in well-known laboratory manuals, including Ausubel et al., eds., Current Protocols in Molecular Biology, John Wiley and Sons, NY 1987; and Sambrook et al., eds., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, 1989 (cited on page 11 of Applicants' specification). Applicants have provided working examples in which human cDNA and genomic clones were obtained through the use of sequence information from a mouse clone and conventional cloning techniques.

Polynucleotides encoding TPO are recognized by their degree of sequence identity to known TPO clones and by the biological activity of the encoded protein. The claimed polynucleotides are at least 80% identical in sequence to

the region between nucleotides number 237 and 722 of SEQ ID NO:1. As disclosed on page 18 of the specification, thrombopoietin has a two-domain structure, wherein the amino-terminal region of thrombopoietin forms a cytokine-like or erythropoietin-like domain. See also Table 3 on page 21 of the specification. This cytokine-like domain, which resides within the region bounded by residues 45-206 of SEQ ID NO:2 (encoded by nucleotides 237 to 722 of SEQ ID NO:1), is highly conserved between species. For the Examiner's convenience, an alignment of the mouse and human TPO cDNA sequences (attached hereto as Appendix A) is included with this amendment. This alignment was prepared using analytical methods that are standard in the art.

Determination of polynucleotide sequences is routine in the art. Early, manual methods of sequencing have been supplanted by automated techniques that have made possible the sequencing of a large part of the human genome in a remarkably short time. Thus, hybridizing clones identified by methods taught in Applicants' disclosure can be readily sequenced, and the sequences compared to SEQ ID NO:1. Degree of sequence identity is readily determined by alignment and analysis using well known computer programs.

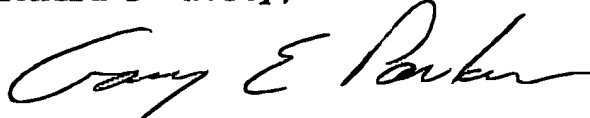
Stimulation of MPL-dependent cell proliferation can be determined as disclosed in Applicants' specification, such as at page 9 and in the Examples (e.g. at page 67).

In view of Applicants' disclosure and the relative skill and knowledge of those in the art, the ordinarily skilled practitioner could readily utilize the sequence information provided by Applicants to clone additional polynucleotides encoding naturally occurring mammalian thrombopoietins having the recited degree of sequence identity and the recited biological activity.

Applicants believe that the amended claims are allowable, and notice to that effect is respectfully requested. If for any reason the Examiner feels that a telephone conference would expedite prosecution of the

application, the Examiner is invited to telephone the undersigned at (206) 442-6673.

Respectfully Submitted,
Richard D. Holly, et al.



Gary E. Parker
Registration No. 31,648

Enclosures:
Appendix A (sequence alignment)

Comparison of:
 (A) zg-htpo-cod.fseq >zg-htpo124
 (B) zg-mtpo-cod.fseq
 using DNA matrix

- 1062 nt
 - 1080 nt

81.1% identity in 1074 nt overlap; init: 2039, opt: 2811

APPENDIX A

```

      10      20      30      40      50      60
zg-htp ATGGAGCTGACTGAATTGCTCCTCGTGGTCATGCTTCTCCTAACTGCAAGGCTAAGGCTG
X:::::::::::::::::: : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt ATGGAGCTGACTGATTTGCTCCTGGCGGCCATGCTTCTTGCACTGGCAAGACTAAGCTG
      10      20      30      40      50      60

      70      80      90     100     110     120
zg-htp TCCAGCCCGGCTCCTCCTGCTTGTGACCTCCGAGTCCTCAGTAACTGCTCCGTGACTCC
:::::::::::: : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt TCCAGCCCGTAGCTCCTGCTGTGACCCCGAGTCTCTAAATAAACTGCTGCGTGACTCC
      70      80      90     100     110     120

      130     140     150     160     170     180
zg-htp CATGTCCTTACAGCAGACTGAGCCAGTGCCAGAGGTTACCCCTTTGCCTACACCTGTC
:: : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt CACCTCCTTACAGCCGACTGAGTCAGTGTCCCGACGTCGACCTTTGTCTATCCCTGTT
      130     140     150     160     170     180

      190     200     210     220     230     240
zg-htp CTGCTGCCTGCTGTGGACTTTAGCTTGGGAGAATGGAAAACCCAGATGGAGGAGACCAAG
:::::::::::::::::: : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt CTGCTGCCTGCTGTGGACTTTAGCTTGGGAGAATGGAAAACCCAGACGGAACAGAGCAAG
      190     200     210     220     230     240

      250     260     270     280     290     300
zg-htp GCACAGGACATTCTGGGAGCAGTGACCTTCTGCTGGAGGGAGTGATGGCAGCACGGGGA
:::::::::::::::::: : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt GCACAGGACATTCTAGGGGAGTGTCCTTCTACTGGAGGGAGTGATGGCAGCACGAGGA
      250     260     270     280     290     300

      310     320     330     340     350     360
zg-htp CAACTGGGACCCACTTGCCTCTCATCCCTCCTGGGCGAGCTTTCTGGACAGGTCCGTCTC
:: : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt CAGTTGGAACCTCCTGCTCTCATCCCTCCTGGGACAGCTTTCTGGGCGAGGTTGCGCTC
      310     320     330     340     350     360

      370     380     390     400     410     420
zg-htp CTCCTTGGGGCCCTGCAGAGCCTCCTTGGTACCCAGCTTCTCCACAGGGCAGGACCAACA
:: : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt CTCCTTGGGGCCCTGCAGGGCCTCTAGGAACCCAGCTTCTCTACAGGGCAGGACCAACA
      370     380     390     400     410     420

      430     440     450     460     470     480
zg-htp GCTCACAGGATCCCAATGCCATCTTCTGAGCTTCCAACACCTGCTCCGAGGAAAGGTG
:::::::::::::::::: : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt GCTCACAGGACCCCAATGCCCTCTTCTTCTGAGCTTGCAACAACCTGCTTCGGGGAAAGGTG
      430     440     450     460     470     480

      490     500     510     520     530     540
zg-htp CGTTTCTGTGCTTGTAGGAGGGTCCACCCCTCTGCGTCAGGCGGGGCCACCCACCACA
:: : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt CGCTTCTGTCTTCTGGTAGAAGGTCCACCCCTCTGTGTCTAGACGGACCTGCCAACCAACA
      490     500     510     520     530     540

      550     560     570     580     590     600
zg-htp GCTGTCCCCAGCAGAACTCTCTAGTCTCTCACTGAACGAGCTCCCAACAGGACTTCT
:::::::::::::::::: : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt GCTGTCCCAAGCAGTACTTCTCAACTCTCTCACTAAACAAGTTCCCAACAGGACTTCT
      550     560     570     580     590     600

      610     620     630     640     650     660
zg-htp GGATTGTTGGAGACAAACTTCACTGCCTCAGCCAGAACTACTGGCTCTGGGCTTCTGAAG
:::::::::::::::::: : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt GGATTGTTGGAGACGAATTCAGTGTCTCAGCCAGAACTGCTGGCCCTGGAATCTCTGAGC
      610     620     630     640     650     660

      670     680     690     700     710
zg-htp TGGCAGCAGGGATTGAGGCCAAGAT---TCCTGGTCTGCTGAACCAAACCTCCAGGTCC
:: : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt AGGCTTCAGGGATTGAGGTCAAGATTACTCTGGTCTGCTGAACCAAACCTCCAGGTCC
      670     680     690     700     710

```

```

      720      730      740      750      760      770
zg-htp CTGGACCAAAATCCCGGATACCTGAACAGGATACACGAACCTCTGAATGGAACCTCGTGG
: : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt CCAGTCCAAATCTCTGGATACCTGAACAGGACACACGGACCTGTGAATGGAACCTCATGGG
      730      740      750      760      770      780

      780      790      800      810      820      830
zg-htp CTCTTTCCTGGACCCTCAGCAGGACCCTAGGAGCCCGGACATTTCTCAGGAACATCA
: : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt CTCTTTCCTGGAACCTCACTTCAGACCCCTGGAAGCCTCAGACATCTCGCCCGGAGCTTC
      790      800      810      820      830      840

      840      850      860      870      880      890
zg-htp GACACAGGCTCCCTGCCACCACCTCCAGCCTGGATATTCTCTTCCCAAGCCATCCT
: : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt AACAAAGGCTCCCTGGCATTCAACCTCCAGGGTGGACTTCTCTTCTCAAGCCTTGCT
      850      860      870      880      890      900

      900      910      920      930      940      950
zg-htp CCTACTGGACAGTATACGCTCTTCCCTCTTCCACCCACCTTGCCCACT-CCTGTGG----
: : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt CCTGATGGAC---ACACACCTTCCCTCTTCCCTGCTTGCCCACTCCCATGGATCT
      910      920      930      940      950      960

      960      970      980      990      1000
zg-htp ----TCCAGCTCCACCCCTGCTTCTGACCTTCTGCTCCAACGCCACCCCTACCAGC
: : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt CCACCCAGCTCCACCCCTGTTTCTGACCTTCCACCATGCCTAACTCTACCGCC
      970      980      990      1000      1010      1020

      1010      1020      1030      1040      1050      1062
zg-htp CCTCTTCTAAACACATCCTACACCCACTCCAGAACTGTCTCAGGAAGGGTAA
: : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
>zg-mt CCTCATCCAGTCACAATGTACCCTCATCCAGGAATTTGTCTCAGGAACATAG
      1030      1040      1050      1060      1070      1080

```